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RESEARCH ARTICLES

Identification of Novel Regions of Allelic Loss From a Genomewide Scan of Esophageal Squamous-Cell Carcinoma in a High-Risk Chinese Population

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Esophageal cancer is one of the most common fatal cancers worldwide. Deletions of genomic regions are thought to be important in esophageal carcinogenesis. We conducted a genomewide scan for regions of allelic loss using microdissected DNA from 11 esophageal squamous-cell carcinoma patients with a family history of upper gastrointestinal tract cancer from a high-risk region in north central China. Allelic patterns of 366 fluorescently labeled microsatellite markers distributed at 10-cM intervals over the 22 autosomal chromosomes were examined. We identified 14 regions with very high frequency (≥ 75%) loss of heterozygosity (LOH), including broad regions encompassing whole chromosome arms (on 3p, 5q, 9p, 9q, and 13q), regions of intermediate size (on 2q, 4p, 11p, and 15q), and more discrete regions identified by very high frequency LOH for a single marker (on 4q, 6q, 8p, 14q, and 17p). Among these 14 regions were 7 not previously described in esophageal squamous-cell carcinoma as having very high frequency LOH (on 2q, 4p, 4q, 6q, 8p, 14q, and 15q). The very high frequency LOH regions identified here may point to major susceptibility genes, including potential tumor suppressor genes and inherited gene loci, which will assist in understanding the molecular events involved in esophageal carcinogenesis and may help in the development of markers for genetic susceptibility testing and screening for the early detection of this cancer. *Genes Chromosomes Cancer 27:217–228, 2000.* †Published 2000 Wiley-Liss, Inc.

INTRODUCTION

Esophageal cancer (EC) is one of the most common fatal cancers worldwide (Boring et al., 1991; Parkin et al., 1993; Berrino et al., 1995). There is great geographic variation in the occurrence of this tumor, including exceptional high-risk areas in northern Iran (Mahboubi et al., 1973), the central Asian republics of the former Soviet Union (Zaridze et al., 1992), South Africa (Jaskiewicz et al., 1987), and northern China, where esophageal squamous-cell carcinoma is the second most common cause of death (Li et al., 1980; Li, 1982).

Epidemiologic studies show that tobacco and alcohol are the major risk factors in the low-risk regions of Europe and North America, but the dominant etiologic exposures in high-risk areas such as Shanxi Province, a region in north central China with some of the highest rates of esophageal cancer in the world, remain unclear. Several environmental etiologic possibilities, including nitrosamines, nutritional deficiencies, fermented and moldy foods, and the inhalation of polycyclic aromatic hydrocarbons, have been considered but have not been convincingly linked to this region's high rates of EC (Yang, 1980).

In addition to environmental factors, there are at least four lines of evidence supporting a role for genetic susceptibility to EC in the high-risk populations of north central China. These include the association of a positive family history with increased risk of developing this cancer (Hu et al., 1991; Wang et al., 1993); evidence of familial aggregation of the disease (Li and He, 1986; Wu et al., 1989; Hu et al., 1992); segregation analyses among high-risk family pedigrees suggesting an autosomal recessive Mendelian pattern of inheritance (Carter et al., 1992); and cytogenetic studies that have found that chromosomal instability, as measured by increased chromosomal aberration rates, fragile sites, and/or sister chromatid exchanges, is much more common in EC cases and their healthy relatives than in healthy persons from families without a history of EC (Wu et al., 1989).

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For a better understanding of the role of genetics in the etiology of EC in north central China and to identify potential susceptibility genes for this cancer, we conducted a genomewide scan to look for regions of allelic loss in 11 cases of EC. The tumors came from patients with a family history of upper gastrointestinal tract cancer from Shanxi Province, a high-risk region in which previous studies have suggested a strong tendency toward familial aggregation. Both tumor and normal tissues were microdissected, and allelic patterns of 366 fluorescently-labeled microsatellite markers distributed at 10-cM intervals over the *22* autosomal chromosomes were examined.

MATERIALS AND METHODS

Patient Selection

Patients presenting to the Shanxi Cancer Hospital in Taiyuan, Shanxi Province, China, who were diagnosed with EC and were considered candidates for curative surgical resection were identified and recruited to participate in this study. The study was approved by the Institutional Review Boards of the Shanxi Cancer Hospital and the U.S. National Cancer Institute. After signed informed consent was obtained, subjects were interviewed to obtain information on demographic and cancer risk factors (e.g., smoking, alcohol, family history, diet). For this initial phase of the study, a total of 12 patients were selected based on the following four criteria: histologic diagnosis of squamous-cell carcinoma of the esophagus by the Shanxi Cancer Hospital and confirmed by NCI; no prior therapy; a positive family history of upper gastrointestinal cancer (EC and/or gastric cardia cancer in one or more first-, second-, or third-degree relatives); and ancestral home in Shanxi Province. Esophageal and gastric cardia cancers were combined for family history because historically both were diagnosed based on dysphagia, and also because their coexistence at very high rates in the same geographic region strongly suggests common environmental and genetic etiologies. Data are shown for only 11 patients because the DNA from 1 patient consistently failed to amplify in polymerase chain reactions.

Biologic Specimen Collection and Processing

Ten ml of venous blood was taken from each patient prior to surgery, and genomic DNA was extracted and purified at the Shanxi Cancer Hospital using routine procedures. Tumor and adjacent normal tissue obtained during surgery were fixed in ethanol and embedded in paraffin by the Department of Pathology of the Shanxi Cancer Hospital according to standard procedures.

Microdissection and Extraction of DNA

Tumor and nontumor cells were microdissected under direct light microscope visualization using methods previously described (Zhuang et al., 1995; Emmert-Buck et al., 1996; Bonner et al., 1997). Briefly, unstained ethanol-fixed, paraffin-embedded 5 μm histologic tissue sections were prepared on glass slides, deparaffinized twice with xylene, rinsed twice with 95% ethanol, stained with eosin, and air-dried. Specific cells of interest were selected from the eosin-stained slides and microdissected either manually or by laser capture microdissection. Procured cells were immediately resuspended in an 80-μl solution containing 0.01 M Tris-HCl, 1 mM EDTA, 1% Tween-20, and 0.1-mg/ml proteinase K (pH 8.0), and incubated for two nights at 37°C. The mixture was boiled for 5 min to inactivate the proteinase K. Two microliters of this solution were used for each PCR reaction.

Markers, PCR, and LOH Reading and Interpretation

Polymorphic microsatellite markers labeled with fluorescein were used for screening, including 366 markers covering all *22* autosomal chromosomes [Cooperative Human Linkage Center (CHLC) Human Screening Version 8, Research Genetics, Huntsville, AL]. In Human Screening Set 8, these markers are spaced every 10 cM, have a mean heterozygosity of 76%, and contain 89% tri- and tetranucleotides.

DNA from microdissected tumor cells and two normal controls (genomic DNA from venous blood and DNA from microdissected lymphocytes and normal stromal cells in the slides from the surgical resection) were used for each patient. PCR reactions were carried out using a 10 μl final volume containing 1.0 μl of 10 × PCR buffer II (100 mM Tris-HCL, pH 8.3, 500-mM KCl), 1.0 μl of 1.25 mM dNTP, 0.6 μl of $MgCl_2$, 2 μl of DNA extraction buffer, 0.6 μl of each primer, and 0.09 μl of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA). The amplifications were performed using a Techne/Genius thermal cycler for 30 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. An elongation step at 72°C for 10 min was added to the final cycle. The PCR products were mixed with 5 μl of formamide loading dye (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol) and were denatured for 6 min at 95°C and chilled on ice until loaded onto a 6%

TABLE 1. Summary of Demographic and Medical Characteristics of Patients and Microsatellite Alterations of 11 Individual Tumors

Case number	ID number	Age/ sex	Tumor location	Lymph node metastasis	Family history of cancer[a]	Tobacco use	Alcohol[b] consump-tion	Pickled vegetable[c] consump-tion	Hot food[d] consump-tion	FAL[e] index	MSI[f]
1	21	57/F	Middle	N	2 EC (father, paternal grandfa-ther)	N	Weekly	Daily	Daily	0.64 (25/39)	3
2	27	59/M	Middle	N	CC (brother)	Y	Daily	Seldom	Seldom	0.53 (20/38)	6
3	80	39/F	Middle	N	EC (father)	N	Never	Daily	Seldom	0.70 (26/37)	6
4	83	64/M	Lower	N	EC (father); cervix Ca (mother)	Y	Weekly	Never	Never	0.62 (24/39)	1
5	93	55/M	Middle	N	EC (father); breast Ca (maternal aunt)	N	Never	Seldom	Daily	0.84 (32/38)	2
6	98	53/M	Upper	N	EC (paternal grand-mother)	Y	Weekly	Daily	Never	0.82 (32/39)	1
7	108	47/M	Middle	Y	EC (paternal uncle); CC (paternal uncle)	Y	Never	Daily	Never	0.70 (26/37)	1
8	109	59/F	Middle	Y	EC (paternal uncle)	N	Daily	Never	Daily	0.74 (29/39)	4
9	113	43/F	Middle	N	EC (maternal uncle)	N	Never	Seldom	Seldom	0.67 (26/39)	3
10	123	56/M	Middle	Unk	2 EC (paternal uncle, paternal grandfa-ther); liver Ca (paternal uncle)	Y	Never	Daily	Seldom	0.77 (30/39)	2
11	186	43/M	Middle	N	EC (paternal uncle); CC (brother)	N	Weekly	Daily	Never	0.56 (22/39)	5

[a]EC = esophageal cancer; CC = cardia cancer.
[b]Traditional Chinese liquor containing ≥40% ethanol.
[c]Turnip greens, Chinese cabbage, or other vegetables preserved in water without refrigeration for several months during the winter.
[d]Foods at very hot temperature.
[e]Fractional allelic loss.
[f]Number of markers with microsatellite instability.

TABLE 2. Frequency of Allelic Loss on Each Chromosome Arm

Chromosome arm	% LOH (total number of markers with LOH in all cases divided by the total number of informative markers in all cases)[a]	Chromosome arm	% LOH (total number of markers with LOH in all cases divided by the total number of informative markers in all cases)[a]
1p	26 (23/88)	11p	71 (32/45)
1q	24 (22/91)	11q	52 (28/54)
2p	30 (17/57)	12p	24 (10/41)
2q	38 (43/113)	12q	35 (30/85)
3p	89 (65/73)	13q	95 (73/77)
3q	28 (25/90)	14q	32 (29/91)
4p	65 (24/37)	15q	46 (23/50)
4q	67 (62/93)	16p	38 (5/13)
5p	38 (27/71)	16q	19 (9/47)
5q	79 (77/97)	17p	69 (22/32)
6p	29 (8/28)	17q	64 (16/25)
6q	45 (38/85)	18p	50 (7/14)
7p	11 (6/54)	18q	45 (17/38)
7q	23 (19/81)	19p	25 (5/20)
8p	52 (27/52)	19q	22 (6/27)
8q	52 (34/66)	20p	23 (8/35)
9p	81 (34/42)	20q	45 (5/11)
9q	86 (32/37)	21q	42 (14/33)
10p	43 (20/47)	22q	34 (12/35)
10q	38 (24/63)		

[a]Informative markers = markers with ≥5 informative cases (n = 267).

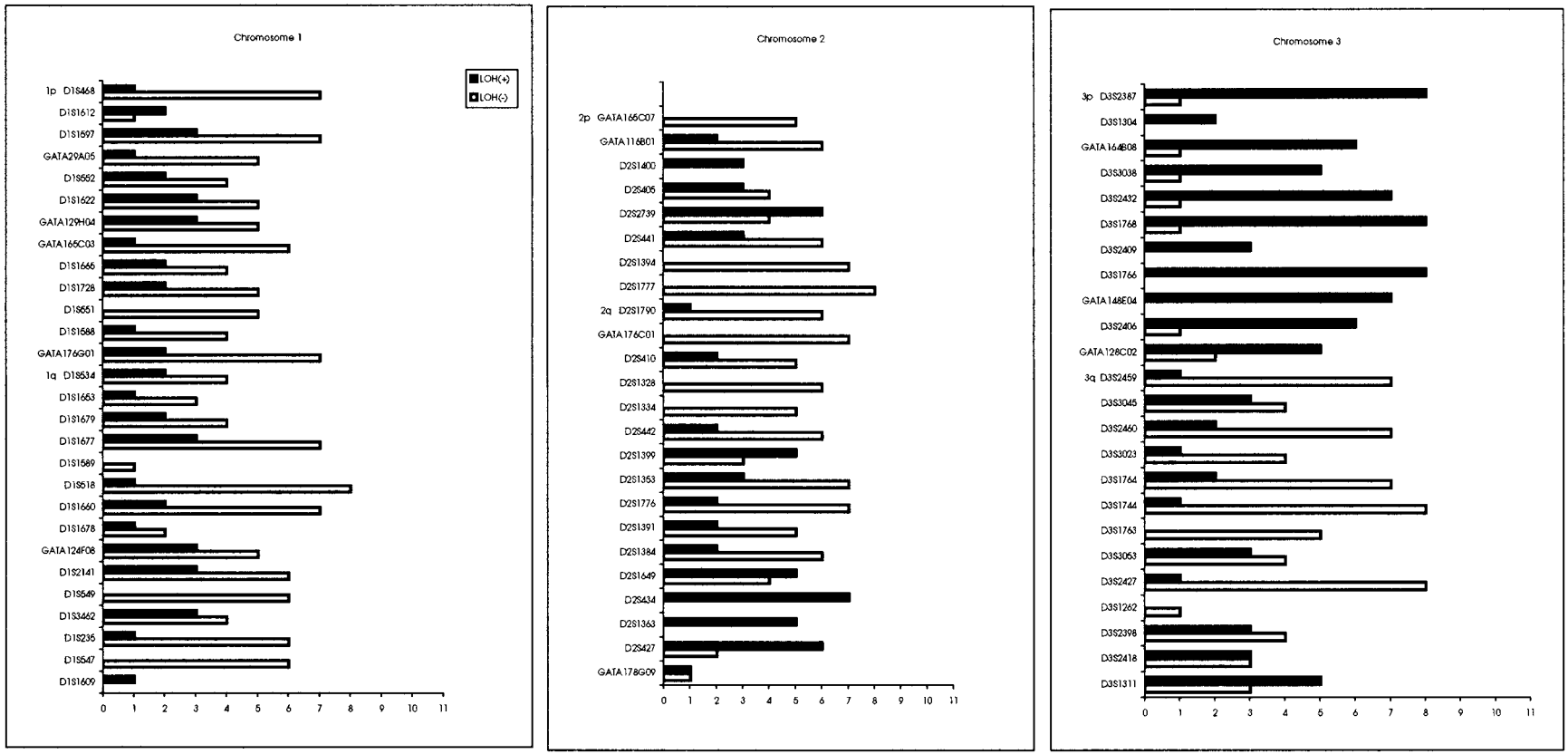
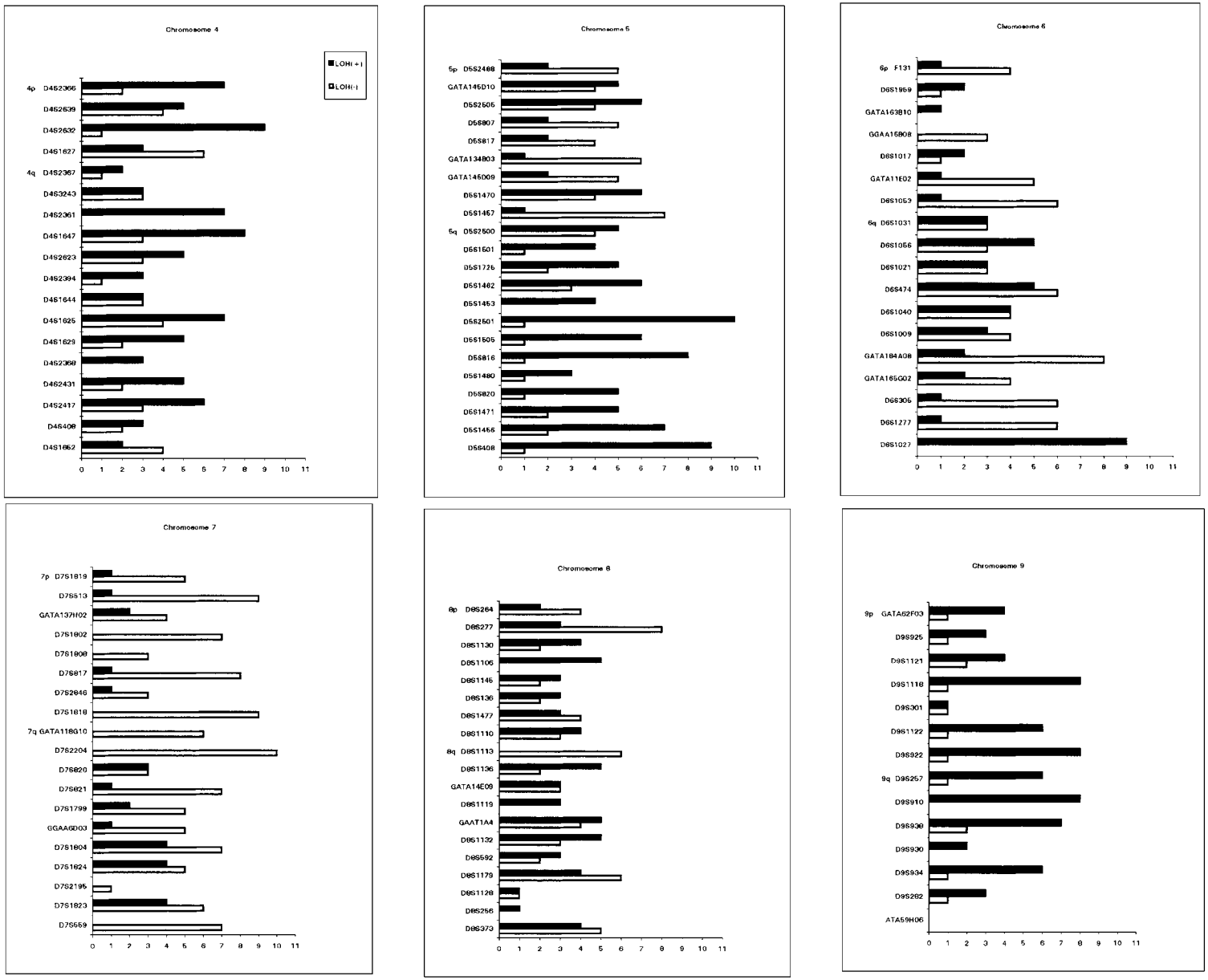


Figure 1. Frequency of LOH for each individual marker. X axis: number of cases; Y axis: marker.



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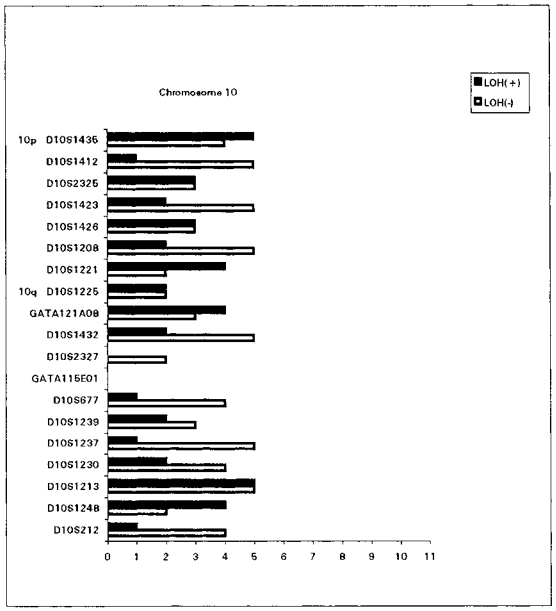

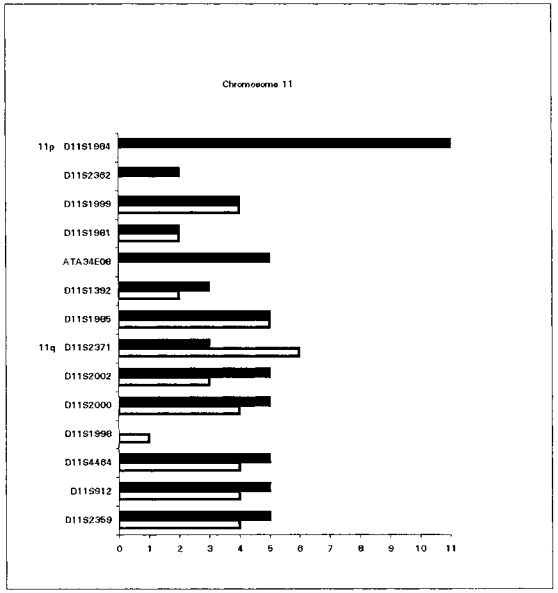

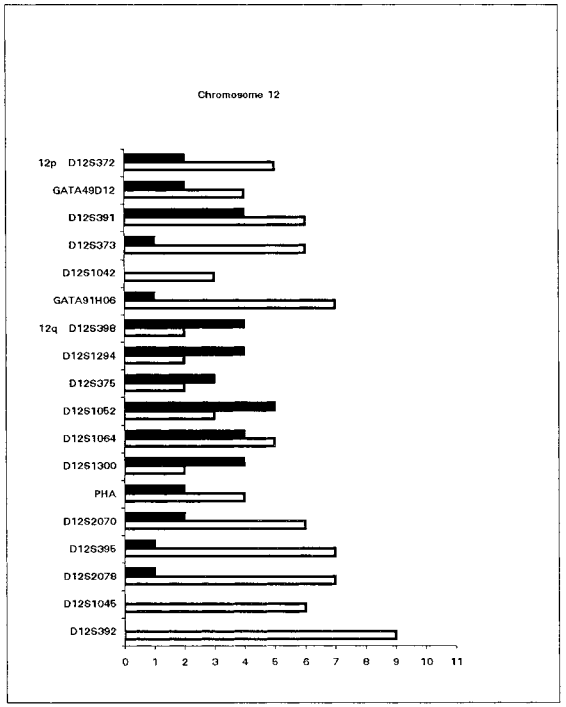

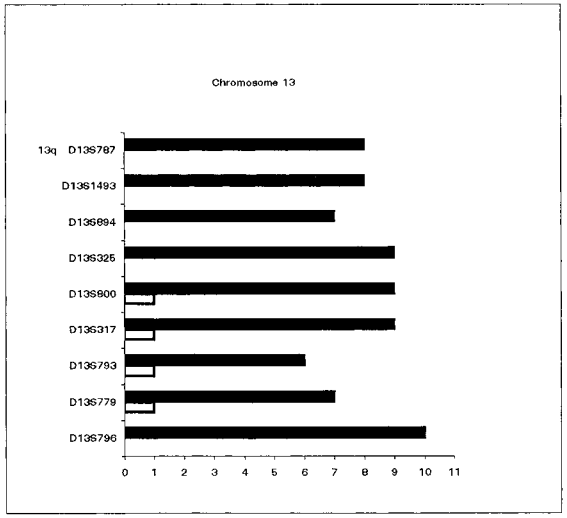

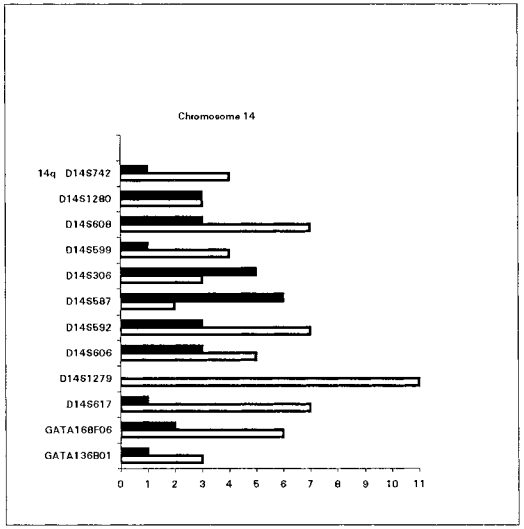

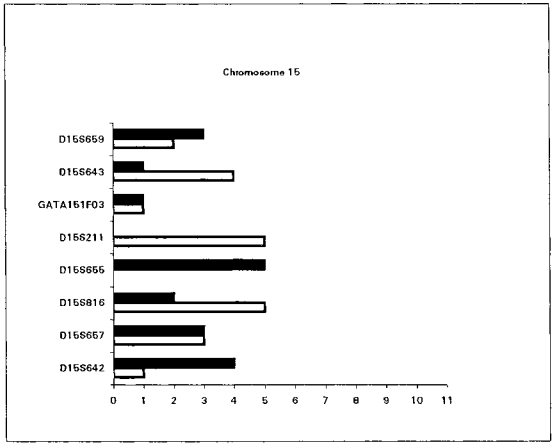

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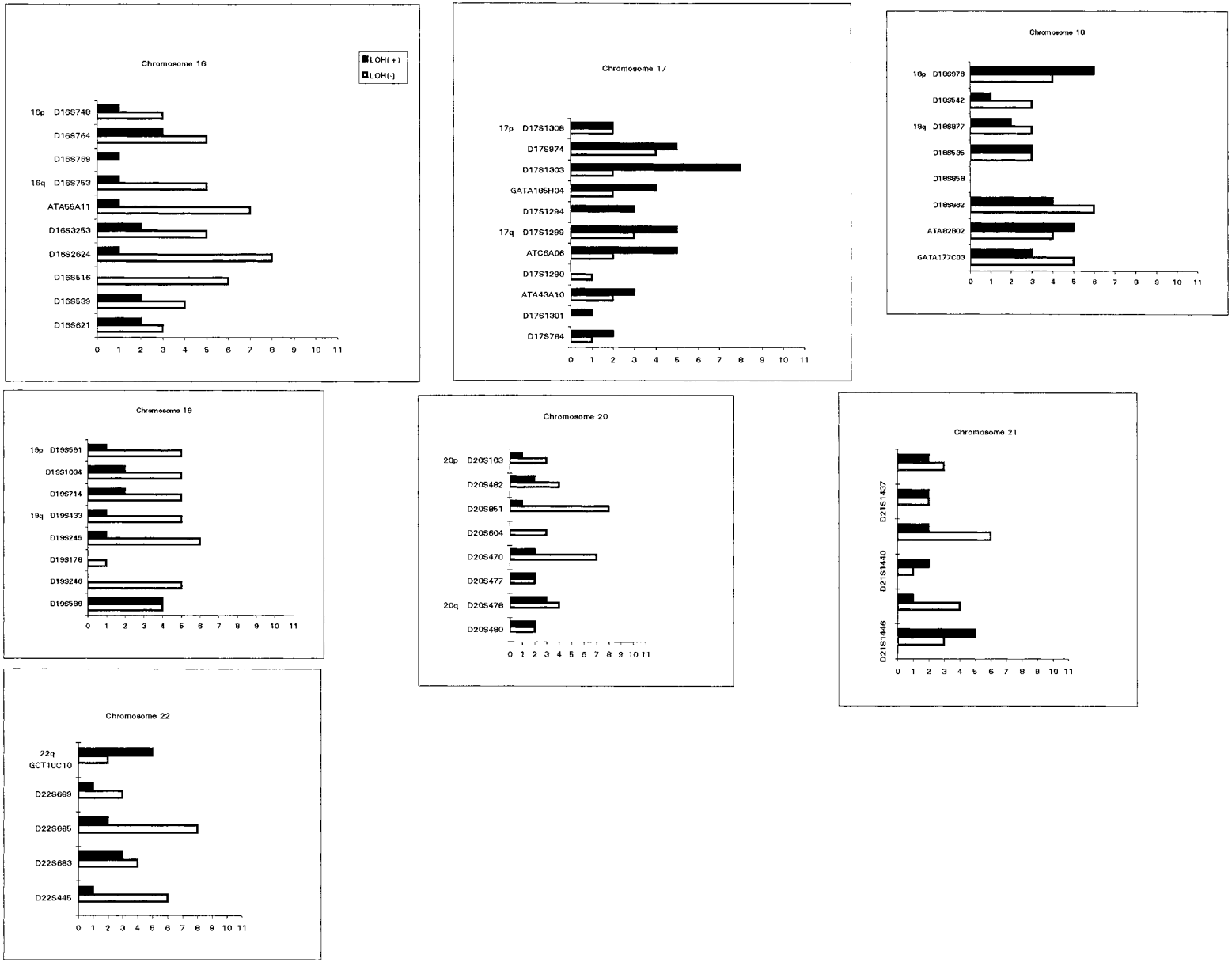

Figure 1. (Continued.)

polyacrylamide gel. Samples were electrophoresed at 25 watts for 1–3 hr.

The gels were scanned by SCANNER (Molecular Dynamics, Model 575; scanner type: FluorImager SI; image analysis software: Image QuanNT, version 3.51, 1995). LOH was defined as visually evident complete or near complete loss of a band in the tumor sample relative to corresponding normal DNA. Microsatellite instability was defined as the presence of an extra band (allele) in the tumor DNA but not in the two corresponding normal DNAs. Convincing evidence of a homozygous deletion in a tumor sample was not observed at any of the 366 markers employed.

Two reviewers independently read all results from computer printouts, and all positives identified by either of the two initial readers were confirmed by a third reader.

Calculation of Frequency of Allelic Loss

The frequency of allelic loss was calculated for each tumor, for each chromosome arm, and for each individual marker. The frequency of allelic loss in each tumor was determined as the fractional allelic loss (FAL) index (Vogelstein et al., 1989). The FAL was calculated as the number of chromosome arms in a tumor showing any LOH divided by the total number of chromosome arms in the tumor having any informative markers.

The frequency of allelic loss on each chromosome arm was calculated as the total number of markers in all tumors showing LOH on the arm divided by the total number of informative markers in all tumors on the arm.

The frequency of allelic loss at each individual marker was determined for each marker in which ≥ 5 of the 11 tumors were informative, and was calculated as the number of tumors with allelic loss at the marker divided by the number of informative tumors at the marker. The frequency of allelic loss at individual markers was classified as low (0%–24%), medium (25%–49%), high (50%–74%), or very high ($\geq$ 75%).

Grouping of Very High Frequency Allelic Loss Loci into Regions

In order to identify the chromosome regions of greatest interest, we reviewed the LOH data for the very high frequency LOH markers (as defined) and attempted to group these markers further as broad regions (i.e., LOH on entire chromosome arms), regions of intermediate size, and discrete regions (i.e., single markers).

RESULTS

Characteristics of Patients

A total of 11 esophageal squamous-cell carcinoma patients were analyzed, including 7 males and 4 females. Ten of the patients had first- or second-degree relatives diagnosed with EC, and one had a brother with gastric cardia cancer. Of the seven male patients, five smoked and five reported drinking Chinese liquor. Of the four female patients, none smoked and two reported drinking Chinese liquor. Forty-five percent of the patients reported eating pickled vegetables frequently, whereas only 27% said that they ate hot food frequently (Table 1).

Frequency of Allelic Loss and Microsatellite Instability in Each Tumor

The FAL index ranged from 53% to 84% (Table 1). Microsatellite instability was observed in all 11 cases, but the number of markers per case showing instability was low, ranging from 1 to 6. Only one individual marker showed instability in more than one case (Table 1).

Frequency of Allelic Loss on Each Chromosome Arm

The frequency of LOH on each chromosome arm ranged from 11% to 95%. As shown in Table 2 for the 267 markers with ≥ 5 informative cases, very high frequencies of allelic loss were observed on 3p (89%), 5q (79%), 9p (81%), 9q (86%), and 13q (95%), whereas high frequencies of allelic loss were observed on 4p (65%), 4q (67%), 8p (52%), 8q (52%), 11p (71%), 11q (52%), 17p (69%), 17q (64%), and 18p (50%).

Frequency of Allelic Loss at Individual Markers

Forty-three of the 366 markers failed to amplify in any of the 11 patients. Figure 1 shows the number of informative patients, the number with LOH, and the number without LOH for each of the 323 individual markers that could be amplified and tested. Of the 267 informative markers (those with ≥ 5 informative cases), 76 showed a low frequency of allelic loss per marker, 72 had a medium frequency, 73 had a high frequency, and 46 had a very high frequency of allelic loss per marker. An example of very high frequency allelic loss, at the D13S796 locus, is shown in Figure 2.

Table 3 shows the chromosomal location, marker name, and LOH information for the 46 informative markers with a very high frequency of LOH ($\geq$ 75%). These markers were found on 14 different chromosomal regions, including 2q (3 markers), 3p

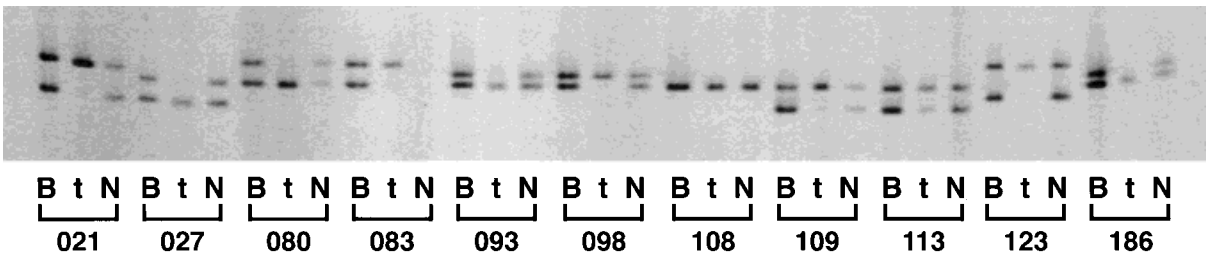

Figure 2. LOH analyses at the D13S796 locus. Analyses of DNA from blood (B), tumor tissue (t), and nontumor tissue (N). Ten of 11 cases are informative and LOH-positive. Case 108 is homozygous.

(8 markers), 4p (2 markers), 4q (1 marker), 5q (7 markers), 6q (1 marker), 8p (1 marker), 9p (4 markers), 9q (4 markers), 11p (2 markers), 13q (9 markers), 14q (1 marker), 15q (2 markers), and 17p (1 marker). These included some broad regions encompassing whole chromosome arms (3p, 5q, 9p, 9q, and 13q), some regions of intermediate size, where the apparent smallest common region that was deleted ranged from 24–48 cM (on 2q, 4p, 11p, and 15q), and some more discrete regions identified as very high frequency LOH for a single marker (on 4q, 6q, 8p, 14q, and 17p). Sixteen markers from these 14 regions were reexamined using laser microdissected tumor and nontumor DNA, and the very high frequency of LOH in these regions was confirmed.

DISCUSSION

EC remains a common fatal cancer worldwide, and new methods to avert its morbidity and mortality through strategies aimed at prevention and control are a high priority. Such efforts include the identification of major susceptibility genes that might focus prevention and early detection measures on very high risk individuals. The current study represents the first step in our efforts to identify major genes for EC and to learn about other molecular events involved in esophageal carcinogenesis that might facilitate the development of prevention and early detection strategies.

The importance of genetic factors in the development of esophageal squamous-cell carcinoma has been highlighted by molecular studies on human EC that have revealed frequent abnormalities in several genetic loci, including both oncogenes and tumor suppressor genes. These abnormalities include amplification of the cellular oncogenes for *MYC*, the epidermal growth factor receptor gene, growth factor genes *INT2* and *HST1*, and the cell cycle-regulatory gene *CYCLIND1* (*PRAD1*) (Montesano et al., 1996). Notably lacking are abnormalities in the *RAS* oncogene. LOH has been reported in *TP53* (Montesano et al., 1996; Shimada et al., 1996), *RB1*, *BRCA1*, *APC*, *MCC*, *CDKN2A*, and *IRF1* genes, and in a putative tumor suppressor gene in the region of 3p13–26 (Montesano et al., 1996; Ogasawara et al., 1996; Wang et al., 1996; Zou et al., 1997). Allelotype analyses have also been reported (Aoki et al., 1994; Montesano et al., 1996). Ohta et al. (1996) also reported aberrant transcripts without point mutations from the newly identified tumor suppressor gene *FHIT* in EC cases. Taken together, these data suggest that oncogene amplifications, allelic deletions, and other molecular abnormalities are common events in the formation and progression of most esophageal squamous-cell carcinomas, and that the accumulation of multiple allelic deletions involving specific tumor suppressor genes may be important in esophageal tumorigenesis and tumor evolution.

Although a number of molecular studies on EC from high-risk areas of China have recently appeared (Lu et al., 1988; Bennett et al., 1991, 1992; Huang et al., 1993; Jiang et al., 1993; Xiao et al., 1993; Zhu et al., 1993; Aoki et al., 1994; Lung et al., 1996; Wang et al., 1996; Bennett et al., 1997), most of these have focused on amplification of oncogenes, and only four have used LOH techniques (Huang et al., 1993; Xiao et al., 1993; Aoki et al., 1994; Wang et al., 1996).

The current study is the most dense genomewide scan for allelic loss that has been performed in patients with esophageal squamous-cell carcinoma. We used nearly sevenfold more markers than have been reported in previous studies of this tumor. Indeed, although genomewide searches for LOH were recently reported with 184 markers for breast cancer (Kerangueven et al., 1997) and with 275 markers for hepatocellular carcinoma (Boige et al., 1997), we are unaware of reports of tumor DNA scans at any cancer site using 366 markers (10-cM density) as employed in our study.

The present study is also the first genomewide scan of EC to use microdissection to isolate tumor

TABLE 3. Markers With Very High Frequency LOH*

Number	Chromosomal location	Marker	Number tumors with allelic loss/number informative tumors/ number total tumors[a] (LOH%)
1	2q35	D2S434	7/7/11 (100)
2	2q36	D2S1363	5/5/11 (100)
3	2q37	D2S427	6/8/10 (75)
4	3p26	D3S2387	8/9/10 (89)
5	3p24.3–p25	GATA164B08	6/7/11 (86)
6	3p23–p24	D3S3038	5/6/8 (83)
7	3p21.2–p23	D3S2432	7/8/10 (88)
8	3p22–p24	D3S1768	8/9/11 (89)
9	3p14–p21	D3S1766	8/8/11 (100)
10	3p14.1–p14.2	GATA148E04	7/7/9 (100)
11	3p11.2–p13	D3S2406	6/7/7 (86)
12	4p16	D4S2366	7/9/11 (78)
13	4p12–p14	D4S2632	9/10/11 (90)
14	4q21.3–q22	D4S2361	7/7/11 (100)
15	5q13–q14	D5S1501	4/5/8 (90)
16	5q21–q23.3	D5S2501	10/11/11 (91)
17	5q31	D5S1505	6/7/11 (86)
18	5q31.2	D5S816	8/9/11 (89)
19	5q33.1	D5S820	5/6/11 (83)
20	5q35.1	D5S1456	7/9/11 (78)
21	5q35.2–q35.3	D5S408	9/10/11 (90)
22	6q21	D6S1027	9/9/11 (100)
23	8p21.2–p23.3	D8S1106	5/5/11 (100)
24	9p23–p24	GATA62F03	4/5/9 (80)
25	9p11–q11	D9S1118	8/9/11 (89)
26	9p12	D9S1122	6/7/10 (86)
27	9p12–q12	D9S922	8/9/11 (89)
28	9q22.1–q22.2	D9S257	6/7/7 (86)
29	9q22.3–q31	D9S910	8/8/11 (100)
30	9q32	D9S938	7/9/11 (78)
31	9q33	D9S934	6/7/10 (86)
32	11p15.3–p15.5	D11S1984	11/11/11 (100)
33	11p14	ATA34E08	5/5/11 (100)
34	13q11–q12.1	D13S787	8/8/11 (100)
35	13q11	D13S1493	8/8/11 (100)
36	13q12.3–q14.2	D13S894	7/7/11 (100)
37	13q14.1–q14.2	D13S325	9/9/11 (100)
38	13q21.2–q22	D13S800	9/10/11 (90)
39	13q22	D13S317	9/10/10 (90)
40	13q31–q32	D13S793	6/7/8 (86)
41	13q32	D13S779	7/8/11 (88)
42	13q32–q34	D13S796	10/10/11 (100)
43	14q12–q13	D14S587	6/8/10 (75)
44	15q22–q23	D15S655	5/5/11 (100)
45	15q26.3	D15S642	4/5/8 (80)
46	17p11.2–p12	D17S1303	8/10/11 (80)

*Markers where there were ≥5 informative cases and LOH frequency was ≥75%.

[a]The number with successful PCR amplifications.

and normal tissue cells before extracting DNA. Microdissection of tumor and normal tissue before DNA extraction should increase the sensitivity of detecting allelic loss in tumor samples by reducing contamination by normal tissue elements. When tumor is admixed with adjacent normal tissue, as is the case in varying degrees whenever microdissection is not used, some allelic bands will be visualized in tumor samples because of contamination by DNA from nontumor cells, even when allelic loss has actually occurred in the tumor. We also increased the detection of allelic loss in our study by using microsatellite markers with high heterozygosity, composed primarily of tri- and tetranucleotide repeats, which should be more sensitive for identifying LOH than the RFLP probes and Southern blot techniques used in previous reports.

We analyzed the frequency of allelic loss in several ways, for completeness and to allow our results to be compared with previous studies. This highlighted the limitations of some calculated indexes when large numbers of genomic markers are evaluated. For example, the FAL index should be expected to increase as the number of allelic markers increases, which reduces its value in dense genomic scans. The most useful analysis for gene discovery should be the frequency of allelic loss by individual marker, which allows identification of hotspots as short as the spacing of the markers.

In our study, we found a very high frequency of LOH in 14 different chromosome regions, ranging in size from whole chromosome arms to single markers. Seven of these regions have been reported previously as areas of frequent LOH in esophageal squamous-cell carcinoma, and are regions that include candidate tumor suppressor genes: 3p (*MLH1*), 5q (*APC/MCC*), 9p (*MTS1*, *IFNA*), 9q (*ESS1*), 11p15 (*KIP2*), 13q (*RB1*), and 17p (*TP53*) (Matsuoka et al., 1996; Montesano et al., 1996; Dolan et al., 1998). In addition, we found seven new regions with a very high frequency of allelic loss, on 2q, 4p, 4q, 6q, 8p, 14q, and 15q, regions where up to 30 genes have been identified. These regions include one oncogene (*FES* on 15q) (Jhanwar et al., 1984), several genes involved in xenobiotic metabolism (*NAT1* and *NAT2* on 8p) (Hickman et al., 1994), *CYP1A1*, *CYP1A2*, *CYP11A*, and *ALDH6* on 15q (Jaiswal et al., 1986, 1987; Sparkes et al., 1991; Hsu et al., 1994), a cell cycle control gene (*CDKN1C* on 11p) (Matsuoka et al., 1996), and a growth factor-related gene (*IGF1R* on 15q) (Francke et al., 1986). The high-frequency LOH region we found on 17p may also be a new region (unrelated to *TP53*) since marker D17S1303 is not sited at *TP53*. This region of loss (17p 11.2–12) was recently identified as a novel site for a putative tumor suppressor gene in esophageal adenocarcinoma (Dunn et al., 1999). However, since many of the regions of loss we observed were quite broad and we have not ex-

plored *TP53* in detail as yet, we cannot exclude the possibility that LOH at D17S1303 is due to a mutation in *TP53*, hence we have not included it as a new region at this time. Our study provided only limited information relevant to the tylosis oesophageal cancer gene (*TOCG*) on 17q (Risk et al., 1994), which has been implicated in squamous esophageal cancer as part of hereditary syndromes involving palmoplantar keratoderma in Western populations. Just three cases were informative for D17S784, which lies just outside the *TOCG* region, but two of these cases did show LOH for this marker. To our knowledge, tylosis has been reported in China in just two families, and neither had a case of EC (Hu et al., 1985).

In summary, we employed a high-density genomewide scan using DNA microdissected from 11 esophageal squamous-cell carcinoma patients from a high-risk Chinese population where a familial inherited disorder is suspected, and we identified 14 regions with very high frequency allelic loss, including 7 regions not previously described. These data provide a stimulus to examine more cases, with and without a family history of upper gastrointestinal cancer, and to examine the identified high-frequency regions more carefully. Such studies may eventually allow us to understand the molecular progression of esophageal carcinogenesis and enable the development of new tools for prevention and early detection of this disease.

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